



TITLE:

Significance of trough monitoring for tacrolimus blood concentration and calcineurin activity in adult patients undergoing primary living-donor liver transplantation.

AUTHOR(S):

Yano, Ikuko; Masuda, Satohiro; Egawa, Hiroto; Sugimoto, Mitsuhiko; Fukudo, Masahide; Yoshida, Yuko; Hashi, Sachiyo; ... Kaido, Toshimi; Uemoto, Shinji; Inui, Ken-Ichi

---

CITATION:

Yano, Ikuko ...[et al]. Significance of trough monitoring for tacrolimus blood concentration and calcineurin activity in adult patients undergoing primary living-donor liver transplantation.. European journal of clinical pharmacology 2012, 68(3): 259-266

ISSUE DATE:

2012-03

URL:

<http://hdl.handle.net/2433/154556>

RIGHT:

The final publication is available at [www.springerlink.com](http://www.springerlink.com); This is not the published version. Please cite only the published version.; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。

## Significance of Trough Monitoring for Tacrolimus Blood Concentration and Calcineurin Activity in

### Adult Patients Undergoing Primary Living-Donor Liver Transplantation

Ikuko Yano,<sup>1,2</sup> Satohiro Masuda,<sup>1</sup> Hiroto Egawa,<sup>3,4</sup> Yoshihiro Sugimoto,<sup>1</sup> Masahide Fukudo,<sup>1</sup> Yuko

Yoshida,<sup>1</sup> Sachiyo Hashi,<sup>1</sup> Atsushi Yoshizawa,<sup>3</sup> Yasuhiro Ogura,<sup>3</sup> Kohei Ogawa,<sup>3</sup> Akira Mori,<sup>3</sup> Toshimi

Kaido,<sup>3</sup> Shinji Uemoto,<sup>3</sup> and Ken-ichi Inui<sup>1,5</sup>

<sup>1</sup>Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto,  
Japan

<sup>2</sup>Department of Clinical Pharmacy and Education, Graduate School of Pharmaceutical Sciences, Kyoto  
University, Kyoto, Japan.

<sup>3</sup>Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

<sup>4</sup>*Present address:* Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical  
University, Tokyo, Japan

<sup>5</sup>*Present address:* Kyoto Pharmaceutical University, Kyoto, Japan

Corresponding author: Ikuko Yano, Ph.D.

Department of Clinical Pharmacy and Education,

Graduate School of Pharmaceutical Sciences,

Kyoto University

Sakyo-ku, Kyoto 606-8501, Japan

TEL.: +81-75-751-3582

FAX: +81-75-751-3205

E-mail address: [iyano@kuhp.kyoto-u.ac.jp](mailto:iyano@kuhp.kyoto-u.ac.jp)

Running title: Tacrolimus pharmacokinetics and calcineurin activity

## Abstract

*Purpose* Tacrolimus pharmacokinetics and calcineurin activity in peripheral blood mononuclear cells

(PBMC) was investigated in adult patients undergoing primary living-donor liver transplantation (LDLT)

in order to clarify the significance of trough monitoring of blood tacrolimus concentration during the early post-transplantation period.

*Methods* Fourteen patients were enrolled in this study, and time-course data after oral administration of a conventional tacrolimus formulation twice a day were obtained at 1 and 3 weeks after transplantation.

The concentration of tacrolimus in whole blood and calcineurin activity in PBMC was measured.

*Results* The apparent clearance of tacrolimus significantly increased at 3 weeks compared with 1 week after transplantation, although the trough concentration did not significantly differ at 1 and 3 weeks after transplantation. The concentration at each sampling time, except at 1 h post-dose, was well correlated with the area under the concentration-time curve from 0 to 12 h ( $AUC_{0-12}$ ). Neither the concentration at the trough time point nor  $AUC_{0-12}$  was correlated with the area under the calcineurin activity-time curve from 0 to 12 h; calcineurin activity at the trough time point, however, was strongly correlated ( $r^2>0.92$ ).

*Conclusions* Trough concentration monitoring was appropriate for routine tacrolimus dosage adjustment in adult LDLT patients. In addition, monitoring of calcineurin activity at the trough time point could be useful to predict the immunological status during the dosing interval of tacrolimus.



**Key words:** LDLT, pharmacodynamics, pharmacokinetics, therapeutic drug monitoring

## Introduction

Tacrolimus, a calcineurin (CN) inhibitor, has been widely used to prevent acute rejections after liver transplantation, and therapeutic drug monitoring (TDM) of this drug is recommended to adjust dosages because of its narrow therapeutic index [1, 2]. Despite the large inter-individual variation in tacrolimus pharmacokinetics, the area under the concentration-time curve (AUC) *versus* the trough blood concentration ( $C_0$ ) has a nearly linear relationship [3, 4], and  $C_0$  of tacrolimus is usually monitored in patients receiving this drug. However, in some cases of renal transplantation, the correlation between  $C_0$  and the AUC of tacrolimus is not adequate [5]. With cyclosporine, another CN inhibitor,  $C_0$  does not correlate well with systemic drug exposure, and blood concentrations at 2 h post-dose ( $C_2$ ) have been shown to be good predictors for the absorption profile [6]. Levy et al. [7] reported that a new monitoring strategy based on  $C_2$  levels was superior to traditional  $C_0$  monitoring for liver transplant recipients in reduction of the incidence and severity of acute rejections. Therefore, the relationship between  $C_0$  and AUC of tacrolimus should be clarified in adult LDLT patients, because the pharmacokinetics of tacrolimus during the early post-transplantation period may fluctuate widely, according to the regeneration of grafted liver [8, 9].

The measurement of CN phosphatase activity in circulating blood is a pharmacodynamic approach for evaluating the immunosuppressive effect of CN inhibitors [10]. We found that tacrolimus and cyclosporine had different inhibitory effects on CN activity in peripheral blood mononuclear cells

(PBMCs) in *de novo* LDLT patients [11]. CN activity at trough time points represents a surrogate predictor for overall CN activity throughout the dosing intervals after cyclosporine administration in LDLT patients [12]. However, there is little information about the relationship between the overall CN activity throughout the dosing intervals and blood tacrolimus concentrations, and CN activity at trough time points, after oral administration of tacrolimus in this population.

This study was designed to evaluate pharmacokinetic profiles in parallel with CN activity in PBMCs at 1 and 3 weeks after LDLT in adult patients. Our aim was to evaluate the relationship between blood tacrolimus concentration at each sampling time and drug exposure during the dosing intervals, as well as CN activity during the dosing intervals, to clarify the significance of trough monitoring of tacrolimus blood concentration during the early post-transplantation phase.

## Patients and methods

### Study design

Patients more than 18 years old and who underwent primary LDLT between November 2007 and February 2009 at the Department of Transplantation and Immunology, Kyoto University Hospital were included in this study. Patients who suffered from fulminant hepatitis or were co-administered medications incompatible with tacrolimus were excluded. The study was discontinued when the administration of tacrolimus was stopped. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected in *a priori* approval by the Kyoto University Graduate School and the Faculty of Medicine Ethics Committee. Written informed consent was obtained from each patient.

Within 12 h after LDLT, we started immunosuppression with a conventional tacrolimus formulation (Prograf®; Astellas Pharma Inc., Tokyo, Japan) and low-dose corticosteroids. The initial oral dose of tacrolimus was 0.05 mg/kg twice daily (0900 hours and 2100 hours), and the dose was adjusted to target blood tacrolimus concentrations according to the trough measurements during the 3 weeks after transplantation. The target  $C_0$  was set between 10 and 15 ng/ml on postoperative days (PODs) 1–7, between 8 and 12 ng/ml on PODs 8–14, and between 6 and 10 ng/ml after POD 15, using a microparticle enzyme immunoassay (MEIA) method with an IMx® analyzer (Abbott Japan Co. Ltd., Tokyo, Japan). Corticosteroids were administered according to a protocol described previously [11].

Blood samples (2 ml) were taken before and at 1, 2, 4, 8, and 12 h after the morning administration on POD 6 or 8 (at 1 week) and POD 20 or 22 (at 3 weeks) in order to evaluate the effects of time after transplantation on pharmacokinetics and pharmacodynamics of tacrolimus. The concentration of tacrolimus in whole blood was measured using three analytical methods: high-performance liquid chromatography with tandem mass spectrometry (LC/MS/MS), as previously reported [13]; the MEIA method; and the enzyme-multiplied immunoassay instrument (EMIT) method, using the Viva-E® System (Siemens Healthcare Diagnostics, Tokyo, Japan). Samples were assayed as soon as possible after blood collection and preserved at -20°C before the assay if necessary.

CN phosphatase activity in PBMCs was measured using the remainder of the blood sample after blood concentration measurements, as an index of the pharmacological effects of tacrolimus. The assay of CN phosphatase activity in PBMCs was performed by use of [ $\gamma$ -phosphorus32] regulatory subunit type II (RII) phosphopeptide as a substrate, according to a procedure described previously [11]. On the day of transplantation, we obtained a blood sample to determine the baseline CN activity (CN<sub>base</sub>) before the administration of tacrolimus in each patient.

### Pharmacokinetic and pharmacodynamic analysis

The AUC from 0 to 12 h (AUC<sub>0-12</sub>) after tacrolimus administration was calculated according to the trapezoidal rule. The highest observed concentration and associated time point were defined as the

maximum drug concentration ( $C_{\max}$ ) and the time at which the maximum concentration occurs ( $t_{\max}$ ), respectively. The apparent clearance ( $CL/F$ ) was calculated by dividing the morning dose on each study day with the  $AUC_{0-12}$ . The area under the CN activity-time curve from 0 to 12 h ( $AUA_{0-12}$ ) after administration was calculated according to the trapezoidal rule. The greatest observed CN inhibition, which caused a nadir of enzyme activity, and its associated time points were defined as the maximum CN inhibition ( $CN_{\text{nadir}}$ ) and the corresponding time ( $t_{\text{nadir}}$ ), respectively. The relationship between the blood tacrolimus concentration and CN activity in PBMCs was analyzed using the following maximum inhibitory effect ( $E_{\max}$ ) model:

$$CN = E_{\max} - E_{\max} \cdot C / (EC_{50} + C),$$

where CN is the CN activity at blood concentration  $C$ ;  $E_{\max}$  is the maximum inhibitory effect attributable to the drug, which is assumed to be the same as the baseline activity; and  $EC_{50}$  is the blood concentration that gives a half-maximal effect. The fixed parameters ( $\theta$ ) for  $E_{\max}$  and  $EC_{50}$ , were estimated by use of the nonlinear mixed effects modeling program NONMEM version 6.2 (ICON Development Solutions, Ellicott City, MD), and inter-individual variability ( $\eta$ ) for  $E_{\max}$  and  $EC_{50}$  and residual variability ( $\epsilon$ ) were assumed to be log-normally distributed [11].

## Statistical analysis

Data are presented as the mean  $\pm$  standard deviation (SD). Statistical analyses were performed using the statistical software package GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA). The statistical significance of differences in mean values between 2 groups was analyzed using the paired *t* test. Pearson's correlation coefficient (*r*) was used to estimate correlations between 2 factors. A value of *P* < 0.05 was considered statistically significant.

## Results

### Measurement of blood tacrolimus concentrations by 3 methods

We routinely measured blood tacrolimus concentrations by the MEIA method and compared these measurements and those obtained using the EMIT assay with LC/MS/MS data. Although the MEIA or EMIT measurements were correlated with those obtained with the LC/MS/MS method,  $r^2$  values were relatively poor in EMIT measurements (Fig. 1). We used LC/MS/MS data in the following analyses, because it is the most reliable method and because the MEIA method using an IMx® analyzer will not be commercially available after 2010.

### Pharmacokinetic and pharmacodynamic profiles of tacrolimus

We included 14 primary LDLT patients in this study. The patients' demographic characteristics are summarized in Table 1. Figure 2 shows the time-course profiles of the blood tacrolimus concentration and CN activity at 1 and 3 weeks after LDLT. Table 2 shows the corresponding pharmacokinetic and pharmacodynamic parameters for tacrolimus. We could not obtain time-course data for 1 patient at 3 weeks after LDLT because of the decease from infection. The blood concentration profile at 3 weeks after LDLT changed gradually compared with that at 1 week, and the blood concentration at 1 h post-dose at 3 weeks was significantly lower than at 1 week (Fig. 2a). The  $AUC_{0-12}$  was significantly smaller at 3 weeks than at 1 week after LDLT ( $P < 0.05$ ), although the morning dose tended to increase from 0.020



mg/kg at 1 week to 0.026 mg/kg at 3 weeks (Table 2). Eleven of 13 patients showed an increase of CL/F at 3 weeks compared with that at 1 week, and this difference was statistically significant (Table 2,  $P < 0.05$ ). Thirteen patients would yield an 82% power to detect a 0.121 l/h/kg difference in CL/F (0.147 *versus* 0.268 l/h/kg at 1 and 3 weeks, respectively) with a significant level of 0.05 (one-tailed test).

The CN activity changed slightly and in parallel with the blood concentration, and returned to the pre-dose levels by 12 h post-dose at both 1 and 3 weeks after LDLT (Fig. 2b). The CN activity at the trough time point ( $CN_0$ ) was suppressed to approximately 65% of the baseline value at 1 week, and  $CN_0$  at 1 and 3 weeks after LDLT were not statistically significant (Table 2; Fig. 2).

### **Correlation between blood concentration and AUC of tacrolimus**

The trough blood concentration before the morning dose ( $C_0$ ) and before the evening dose ( $C_{12}$ ) showed a high correlation with the  $AUC_{0-12}$  (Fig. 3;  $r^2 = 0.767$  to  $0.888$ ), although the correlation of  $C_0$  at 1 week after LDLT was not so high ( $r^2 = 0.530$ ). The blood concentrations at 2, 4, and 8 h post-dose also showed strong correlations with the  $AUC_{0-12}$ , and the correlation with the blood concentration at 1 h post-dose was weak ( $r^2 = 0.411$  or  $0.498$ ).

### **Relationship between blood concentration and CN activity**

The CN activity in PBMCs was inhibited in a concentration-dependent manner by tacrolimus, but the inhibition was not complete even at blood tacrolimus concentrations more than 20 ng/ml (Fig. 4).

Through the application of the  $E_{\max}$  model to data from the 14 patients, we calculated the  $EC_{50}$  as 20.9 ng/ml (95% confident interval: 11.6–30.2) and the  $E_{\max}$  as 64.6 pmol/min/mg protein (95% confident interval: 56.3–72.9) by using the nonlinear mixed effects model.

### **Correlation between tacrolimus exposure and pharmacodynamic response**

Both pharmacodynamic parameters  $CN_0$  and  $AUA_{0-12}$  did not correlate with  $C_0$  (Figs. 5a and b).

The correlation between the  $AUA_{0-12}$  and the  $AUC_{0-12}$  was also weak (Fig. 5c). However, the  $AUA_{0-12}$  showed a strong correlation with  $CN_0$  activity ( $r^2 = 0.919$  or  $0.931$ , Fig. 5d), as did CN activity at other time points (data not shown).

## Discussion

LDLT and subsequent immunosuppressive therapy provide excellent results and are usually used in combination with a deceased donor organ-transplant program [14, 15]. Although the prevention of immunological reactions with sufficient immunosuppression prolongs graft and patient survival rates, the large inter-individual variation in tacrolimus pharmacokinetics interferes with treatment [16]. We previously reported that the probability of acute cellular rejection during the first 10 days after surgery was significantly associated with the average trough concentration of tacrolimus between PODs 2 and 4 [17]. However, we had no clear evidence of the trough monitoring or AUC-based monitoring of tacrolimus blood concentration. In this study, we confirmed that the trough blood concentration was a good surrogate marker for tacrolimus exposure during the dosing interval in LDLT patients.

In the time-course study at 3 weeks after LDLT,  $t_{\max}$  was prolonged and the difference between peak and trough concentrations was smaller compared with that at 1 week, indicating the delayed and poor absorption of tacrolimus at 3 weeks after LDLT (Fig. 2). Although we cannot completely explain this phenomenon, the first-pass effects in the intestine and/or liver by cytochrome 3A and P-glycoprotein may be increased at 3 weeks after LDLT compared with at 1 week. In addition, the apparent clearance of tacrolimus at 3 weeks after LDLT was approximately twice that at 1 week (Table 2). We previously reported that the total-body clearance of tacrolimus in LDLT patients was increased according to the POD because of the regeneration of grafted liver [8, 9]. According to the previously estimated population

pharmacokinetic parameters [8], a typical adult LDLT recipient in this study (grafted liver weight of 611 g, normal hepatic and renal function, and body weight of 67.1 kg during week 1 and 63.5 kg during week 3) would have a CL/F of 0.186 L/h/kg on POD 7 and 0.241 L/h/kg on POD 21; these values are comparable to the mean CL/F in this study.

We examined the correlation between  $AUC_{0-12}$  and blood concentration at each sampling point (Fig. 3). Although the correlation between the blood concentration at 1 h post-dose and the  $AUC_{0-12}$  was weak, other blood concentrations showed reasonable or strong correlations with the  $AUC_{0-12}$ . Since the blood concentration at 12 h post-dose ( $C_{12}$ ) will correlate with  $AUC_{0-12}$  for this dose and that  $C_0$  should correlate with  $AUC_{0-12}$  for the previous dose,  $C_{12}$  showed higher correlation than  $C_0$ . The relatively weak correlation found in  $C_0$  at 1 week after LDLT was considered due to a large variability in the pharmacokinetics of tacrolimus immediately after LDLT, similar to the findings in renal transplant recipients [5]. A good correlation between AUC from 0 to 24 h and trough concentrations for both twice-daily and once-daily prolonged tacrolimus formulations were reported in *de novo* kidney transplantation [18]. According to the previous report by Scholten et al. [5], Bayesian forecasting with a 2-point sampling strategy, a trough level and a second sample obtained between 2 and 4 h post-dose, might improve the correlation with  $AUC_{0-12}$ . Taking these finding into consideration, we concluded that a single-trough concentration before the morning dose is sufficient to provide an index of blood tacrolimus exposure during the dosing interval in LDLT patients, even during the early post-transplantation period.

The CN activity changed in parallel with the blood tacrolimus concentration, and showed a similar flat time profile at 1 and 3 weeks after LDLT (Fig. 2). These results indicated that unlike cyclosporine [12], tacrolimus partially suppressed CN activity throughout the dosing interval, probably because of the limited amount of active FK506-binding protein 12 in PBMCs [19]. The relationship between CN activity and blood tacrolimus concentration was analyzed using an  $E_{\max}$  model with an  $EC_{50}$  of 20.9 ng/mL (Fig. 4), which is higher than the upper limit of the therapeutic range of tacrolimus, as discussed in our previous report [11]. Additionally, Fig. 4 showed a large inter-individual variability in the relation between tacrolimus exposure and CN activity. This phenomenon may be explained by the inter-individual variability of expression level of P-glycoprotein in PBMCs [20] or variation in PBMC subset concentrations [21].

We next examined the correlation between pharmacokinetic and pharmacodynamic parameters. A single  $C_0$  did not correlate with  $CN_0$  or  $AUA_{0-12}$  (Figs. 5a, b). In addition, as in our previous study of cyclosporine [12], the  $AUC_{0-12}$  of tacrolimus showed no relationship with  $AUA_{0-12}$  (Fig. 5c). These results indicate a large inter-individual variability in the relationship between tacrolimus blood exposure and CN activity; monitoring of only tacrolimus blood concentrations does not adequately maintain CN activity at a targeted level, as reported by Blanchet et al. [22]. However,  $CN_0$  activity was strongly correlated with the  $AUA_{0-12}$  (Fig. 5d,  $r^2 > 0.92$ ). We recently reported that CN activity rapidly increased a few days before the onset of acute rejection in 2 patients after living-donor kidney transplantation [23].

Therefore, monitoring CN activity at the trough time point would be useful for predicting the overall CN activity in LDLT patients administered tacrolimus. In this study, we could not clarify the relationship between calcineurin activity and clinical outcomes, nor therapeutic range of calcineurin activity. Significance of measurements of calcineurin activity remains to be clarified in a large cohort of liver transplant recipients.

In conclusion, we have shown that  $C_0$  monitoring of tacrolimus can be used to evaluate drug exposure in LDLT patients during the early post-transplantation period, and that monitoring of CN activity at the trough time point could be useful to predict the immunological status during the dosing interval of tacrolimus.

*Acknowledgment:* The authors would like to thank Junichi Nomura, Takashi Ikoma, and Ikuo Sekiguchi for their kind assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors declare that they have no conflict of interest.

## References

1. O'Grady JG, Burroughs A, Hardy P, Elbourne D, Truesdale A (2002) Tacrolimus versus microemulsified ciclosporin in liver transplantation: the TMC randomised controlled trial. *Lancet* 360(9340):1119-1125. doi:10.1016/S0140-6736(02)11196-2
2. Yasuhara M, Hashida T, Toraguchi M, Hashimoto Y, Kimura M, Inui K, Hori R, Inomata Y, Tanaka K, Yamaoka Y (1995) Pharmacokinetics and pharmacodynamics of FK 506 in pediatric patients receiving living-related donor liver transplantations. *Transplant Proc* 27(1):1108-1110.
3. Jusko WJ, Piekoszewski W, Klintmalm GB, Shaefer MS, Hebert MF, Piergies AA, Lee CC, Schechter P, Mekki QA (1995) Pharmacokinetics of tacrolimus in liver transplant patients. *Clin Pharmacol Ther* 57(3):281-290. doi:10.1016/0009-9236(95)90153-1
4. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, McMichael J, Lever J, Burckart G, Starzl T (1995) Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet* 29(6):404-430. doi:10.2165/00003088-199529060-00003
5. Scholten EM, Cremers SC, Schoemaker RC, Rowshani AT, van Kan EJ, den Hartigh J, Paul LC, de Fijter JW (2005) AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. *Kidney Int* 67(6):2440-2447. doi: 10.1111/j.1523-1755.2005.00352.x

6. Cantarovich M, Barkun JS, Tchervenkov JI, Besner JG, Aspeslet L, Metrakos P (1998) Comparison of  
  
neoral dose monitoring with cyclosporine through levels versus 2-h postdose levels in stable liver  
  
transplant patients. *Transplantation* 66(12):1621-1627.
  
7. Levy G, Burra P, Cavallari A, Duvoux C, Lake J, Mayer AD, Mies S, Pollard SG, Varo E, Villamil F,  
  
Johnston A (2002) Improved clinical outcomes for liver transplant recipients using cyclosporine  
  
monitoring based on 2-h post-dose levels (C2). *Transplantation* 73(6):953-959.
  
8. Fukatsu S, Yano I, Igarashi T, Hashida T, Takayanagi K, Saito H, Uemoto S, Kiuchi T, Tanaka K, Inui  
  
K, Tanaka K, Inui K (2001) Population pharmacokinetics of tacrolimus in adult recipients receiving  
  
living-donor liver transplantation. *Eur J Clin Pharmacol* 57(6-7):479-484. doi:10.1007/s002280100331
  
9. Fukudo M, Yano I, Fukatsu S, Saito H, Uemoto S, Kiuchi T, Tanaka K, Inui K (2003) Forecasting of  
  
blood tacrolimus concentrations based on the Bayesian method in adult patients receiving living-donor  
  
liver transplantation. *Clin Pharmacokinet* 42(13):1161-1178. doi:10.2165/00003088-200342130-00006
  
10. Yano I (2008) Pharmacodynamic monitoring of calcineurin phosphatase activity in transplant patients  
  
treated with calcineurin inhibitors. *Drug Metab Pharmacokinet* 23(3):150-157.  
  
doi:10.2133/dmpk.23.150
  
11. Fukudo M, Yano I, Masuda S, Fukatsu S, Katsura T, Ogura Y, Oike F, Takada Y, Tanaka K, Inui K  
  
(2005) Pharmacodynamic analysis of tacrolimus and cyclosporine in patients of living-donor liver  
  
transplantation. *Clin Pharmacol Ther* 78(2):168-181. doi: 10.1016/j.clpt.2005.04.008



12. Fukudo M, Yano I, Masuda S, Katsura T, Ogura Y, Oike F, Takada Y, Tanaka K, Inui K (2006)

Cyclosporine exposure and calcineurin phosphatase activity in living-donor liver transplant patients:

twice daily vs. once daily dosing. *Liver Transpl* 12(2):292-300. doi: 10.1002/lt.20609

13. Shimomura M, Masuda S, Goto M, Katsura T, Kiuchi T, Ogura Y, Oike F, Takada Y, Uemoto S, Inui

K (2008) Required transient dose escalation of tacrolimus in living-donor liver transplant recipients

with high concentrations of a minor metabolite M-II in bile. *Drug Metab Pharmacokinet* 23(5):313-317.

doi: 10.2133/dmpk.23.313

14. Trotter JF, Wachs M, Everson GT, Kam I (2002) Adult-to-adult transplantation of the right hepatic lobe

from a living donor. *N Engl J Med* 346(14):1074-1082. doi: 10.1056/NEJMra011629

15. Tanaka K, Kiuchi T, Kaihara S (2004) Living related liver donor transplantation: techniques and

caution. *Surg Clin North Am* 84(2):481-493. doi: 10.1016/j.suc.2003.12.006

16. Masuda S, Inui K (2006) An up-date review on individualized dosage adjustment of calcineurin

inhibitors in organ transplant patients. *Pharmacol Ther* 112(1):184-198. doi:

10.1016/j.pharmthera.2006.04.006

17. Masuda S, Goto M, Fukatsu S, Uesugi M, Ogura Y, Oike F, Kiuchi T, Takada Y, Tanaka K, Inui K

(2006) Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in

living-donor liver transplant patients. *Clin Pharmacol Ther* 79(1):90-102. doi:

10.1016/j.clpt.2005.09.013

18. Wlodarczyk Z, Squifflet JP, Ostrowski M, Rigotti P, Stefoni S, Citterio F, Vanrenterghem Y, Krämer BK, Abramowicz D, Oppenheimer F, Pietruck F, Russ G, Karpf C, Undre N (2009) Pharmacokinetics for once- versus twice-daily tacrolimus formulations in de novo kidney transplantation: a randomized, open-label trial. *Am J Transplant* 9(11):2505-2513. doi: 10.1111/j.1600-6143.2009.02794.x
19. Kung L, Halloran PF (2000) Immunophilins may limit calcineurin inhibition by cyclosporine and tacrolimus at high drug concentrations. *Transplantation*. 70(2):327-335.
20. Goto M, Masuda S, Kiuchi T, Ogura Y, Oike F, Tanaka K, Uemoto S, Inui K (2008) Relation between mRNA expression level of multidrug resistance 1/ABCB1 in blood cells and required level of tacrolimus in pediatric living-donor liver transplantation. *J Pharmacol Exp Ther* 325(2):610-616. doi: 10.1124/jpet.107.135665
21. van Rossum HH, Romijn FP, Sellar KJ, Smit NP, van der Boog PJ, de Fijter JW, van Pelt J (2008) Variation in leukocyte subset concentrations affects calcineurin activity measurement: implications for pharmacodynamic monitoring strategies. *Clin Chem*. 54(3):517-524. doi: 10.1373/clinchem.2007.097253
22. Blanchet B, Duvoux C, Costentin CE, Barrault C, Ghaleh B, Salvat A, Jouault H, Astier A, Tod M, Hulin A (2008) Pharmacokinetic–pharmacodynamic assessment of tacrolimus in liver-transplant recipients during the early post-transplantation period. *Ther Drug Monit* 30(4):412-418. doi:10.1097/FTD.0b013e318178e31b

23. Fukudo M, Yano I, Katsura T, Ito N, Yamamoto S, Kamoto T, Ogawa O, Inui K (2010) A transient increase of calcineurin phosphatase activity in living-donor kidney transplant recipients with acute rejection. *Drug Metab Pharmacokinet* 25(5):411-417. doi:10.2133/dmpk.DMPK-10-RG-026

## Figure Legends

**Fig. 1** Correlation between the blood tacrolimus concentration by high-performance liquid

chromatography with tandem mass spectrometry (LC/MS/MS) and microparticle enzyme immunoassay

(MEIA) (a) or enzyme-multiplied immunoassay instrument (EMIT) (b) methods. Each point shows a

time-course datum from 14 patients at 1 week and 13 patients at 3 weeks after living-donor liver

transplantation. Solid and dotted lines show the linear regression line and the line of identity,

respectively

**Fig. 2** Time course of blood tacrolimus concentration and calcineurin (CN) phosphatase activity in

peripheral blood mononuclear cells at 1 week (*open circles*) and 3 weeks (*closed circles*) after living-donor

liver transplantation. Each symbol represents the mean  $\pm$  standard deviation ( $n = 14$  and  $13$  for  $1$  and  $3$

weeks after transplantation, respectively).  $*P < 0.05$ , significantly different from 1 week after

transplantation

**Fig. 3** Correlation between area under the concentration-time curve from 0 to 12 h ( $AUC_{0-12}$ ) and blood

tacrolimus concentration at pre-dose and 1, 2, 4, 8, and 12 h after the morning administration ( $C_0$ ,  $C_1$ ,  $C_2$ ,

$C_4$ ,  $C_8$ , and  $C_{12}$ , respectively). Each point shows a datum from 14 patients at 1 week (*open circles*) and 13

patients at 3 weeks (*closed circles*) after living-donor liver transplantation. Each solid and dashed line

represents the linear regression line at 1 and 3 weeks, respectively

**Fig. 4** Relationship between blood tacrolimus concentration and calcineurin (CN) activity in living-donor liver transplant patients. Each point shows a time-course datum from 14 patients at 1 week (*open circles*) and 13 patients at 3 weeks (*closed circles*) after living-donor liver transplantation. Solid line shows the predicted calcineurin phosphatase activity *versus* the blood tacrolimus concentration profile according to the maximum inhibitory effect model by use of the nonlinear mixed effects modeling program

**Fig. 5** Correlation between pharmacokinetic and pharmacodynamic parameters (a, b, and c) and between pharmacodynamic parameters (d) of tacrolimus in 14 patients at 1 week (*open circles*) and 13 patients at 3 weeks (*closed circles*) after living-donor liver transplantation. Calcineurin activity at the trough time point ( $CN_0$ , panel a) or the area under the calcineurin activity-time curve from 0 to 12 h ( $AUA_{0-12}$ , panel b) *versus* blood concentration pre-dose ( $C_0$ ). Panel c:  $AUA_{0-12}$  *versus* area under the concentration-time curve from 0 to 12 h ( $AUC_{0-12}$ ). Panel d:  $AUA_{0-12}$  *versus*  $CN_0$  activity. Each solid and dashed line represents the linear regression line at 1 and 3 weeks, respectively

**Table 1 Demographic characteristics of the study population**

Characteristic	Number or mean $\pm$ standard deviation
Gender (male/female)	8/6
Age (years)	58 $\pm$ 6
Grafted liver weight (g)	611 $\pm$ 156
GRWR (%)	0.955 $\pm$ 0.184
ABO blood group match	
identical	7
compatible	5
incompatible	2
Primary disease	
Hepatitis B virus infection	3
Hepatitis C virus infection	6
Primary biliary cirrhosis	3
Alcoholic liver cirrhosis	1
Autoimmune hepatitis	1

GRWR, graft-to-recipient weight ratio.

**Table 2 Pharmacokinetic and pharmacodynamic parameters of tacrolimus**

Parameters	Week1 (n = 14)	Week3 (n = 13)
Body weight (kg)	67.1 ± 15.2	63.5 ± 10.1
Morning dose (mg/kg)	0.020 ± 0.015	0.026 ± 0.017
Pharmacokinetic parameters		
C <sub>0</sub> (ng/ml)	9.8 ± 2.6	7.7 ± 2.7
t <sub>max</sub> (h)	2 (1-8)	2 (0-4)
C <sub>max</sub> (ng/ml)	16.0 ± 5.4	12.5 ± 4.9
AUC <sub>0-12</sub> (ng h/ml)	140 ± 38	110 ± 40 <sup>*</sup>
CL/F (l/h/kg)	0.147 ± 0.110	0.268 ± 0.220 <sup>*</sup>
Pharmacodynamic parameters		
CN <sub>base</sub> (pmol/min/mg protein)	73.8 ± 16.2 <sup>a</sup>	- <sup>b</sup>
CN <sub>0</sub> (pmol/min/mg protein)	47.9 ± 14.5	47.0 ± 11.2
t <sub>nadir</sub> (h)	2	4
CN <sub>nadir</sub> (mg/min/mg protein)	40.6 ± 15.4	39.2 ± 9.7
AUA <sub>0-12</sub> (pmol h/min/mg protein)	537 ± 188	512 ± 114

Data are given as the mean ± SD or median (minimum-maximum). <sup>a</sup>n = 10. <sup>b</sup>Only measured on the day

of transplantation. <sup>\*</sup>Significantly different from the mean value at week 1 (*P* < 0.05).

C<sub>0</sub>, blood concentration pre-dose; C<sub>max</sub>, maximum blood concentration; t<sub>max</sub>, time corresponding to C<sub>max</sub>;

AUC<sub>0-12</sub>, area under the concentration-time curve from 0 to 12 h; CL/F, apparent clearance; CN<sub>base</sub>,

calcineurin activity at baseline measured before tacrolimus administration on the day of transplantation;

CN<sub>0</sub>, calcineurin activity pre-dose; CN<sub>nadir</sub>, calcineurin activity at maximum inhibition; t<sub>nadir</sub>, time

corresponding to CN<sub>nadir</sub>; AUA<sub>0-12</sub>, area under the calcineurin activity-time curve from 0 to 12 h.

Fig.1

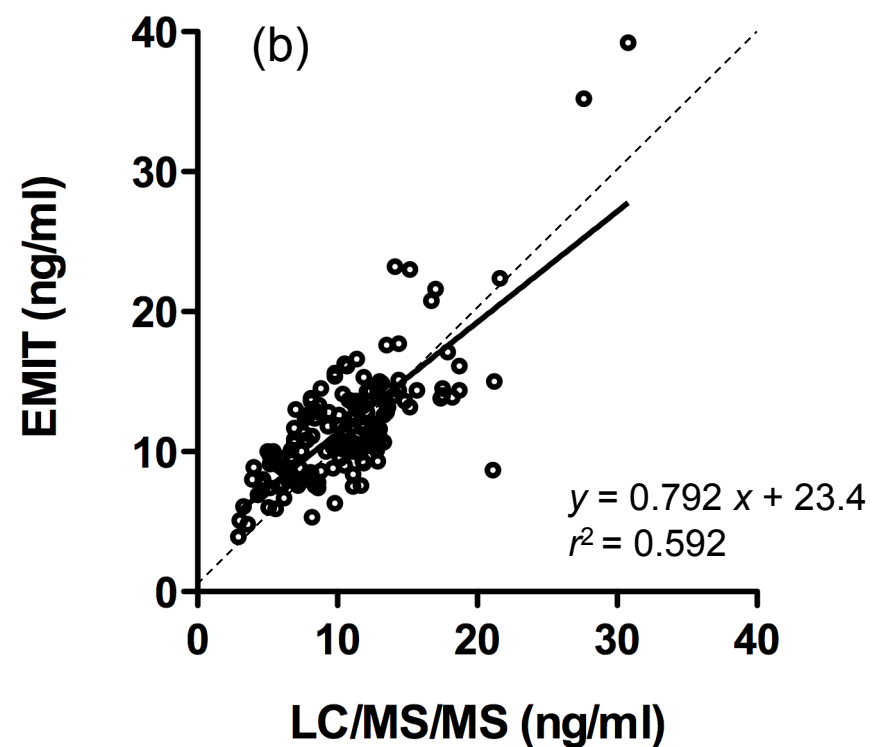
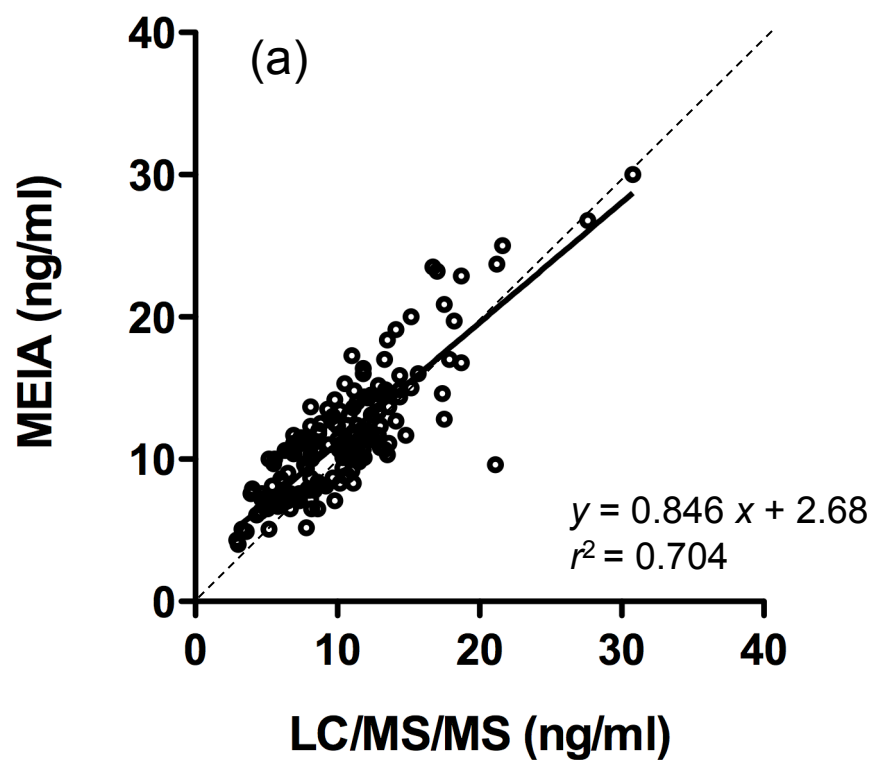




Fig. 2

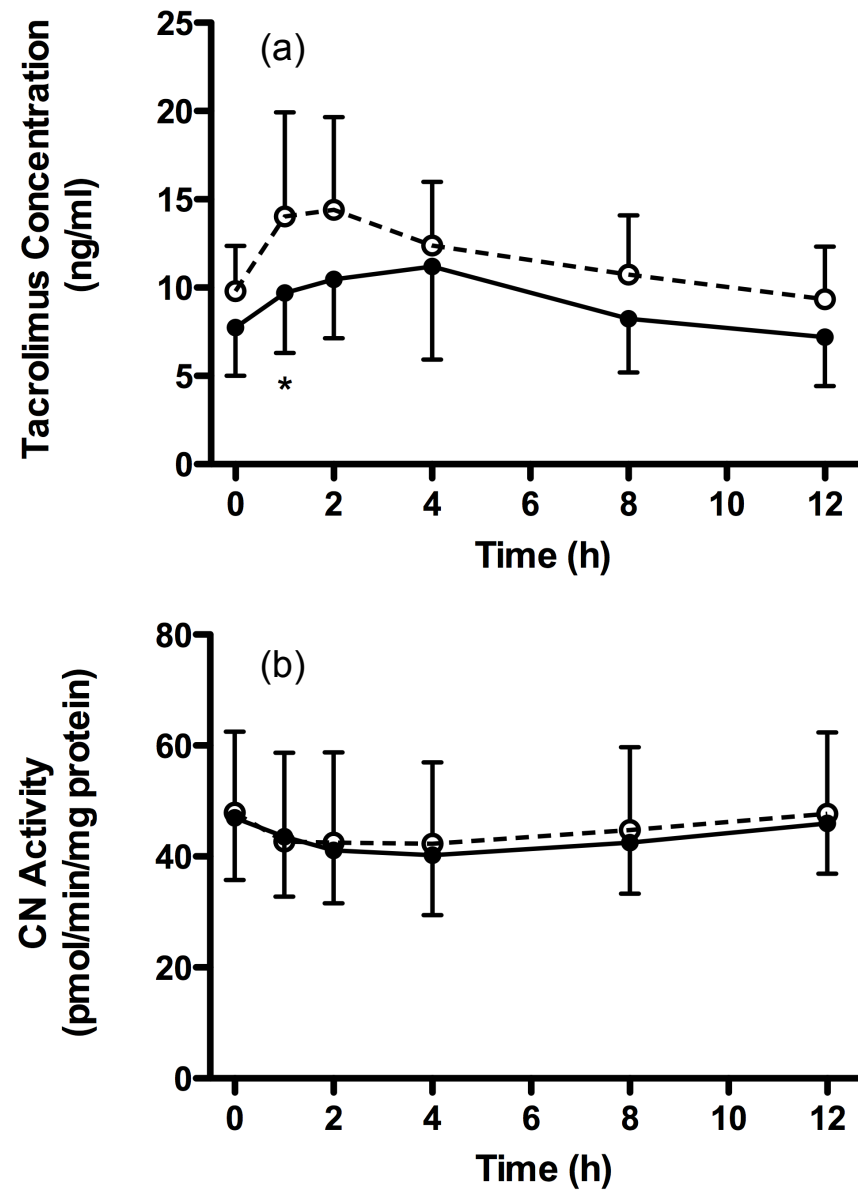


Fig.3

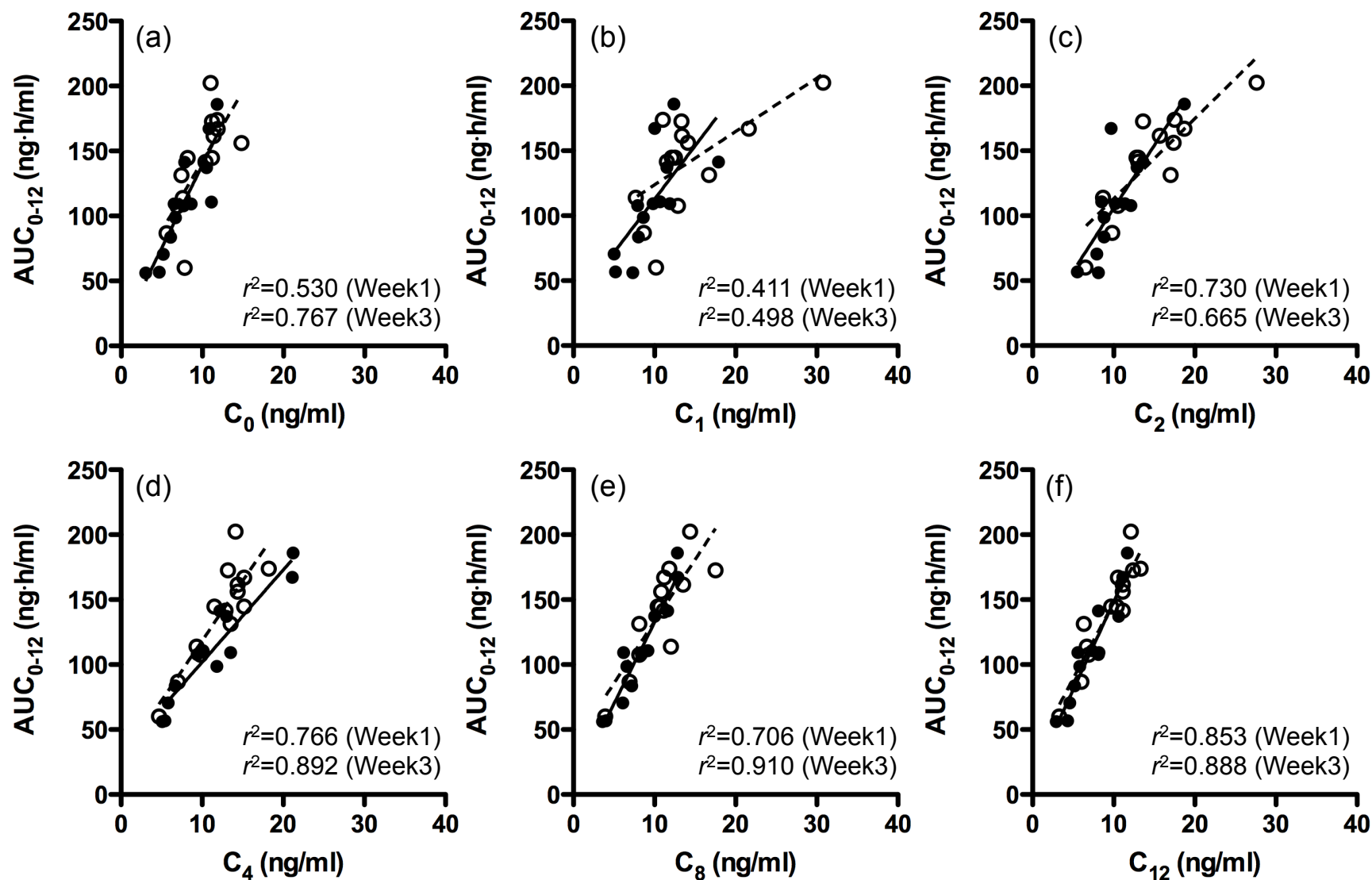


Fig.4

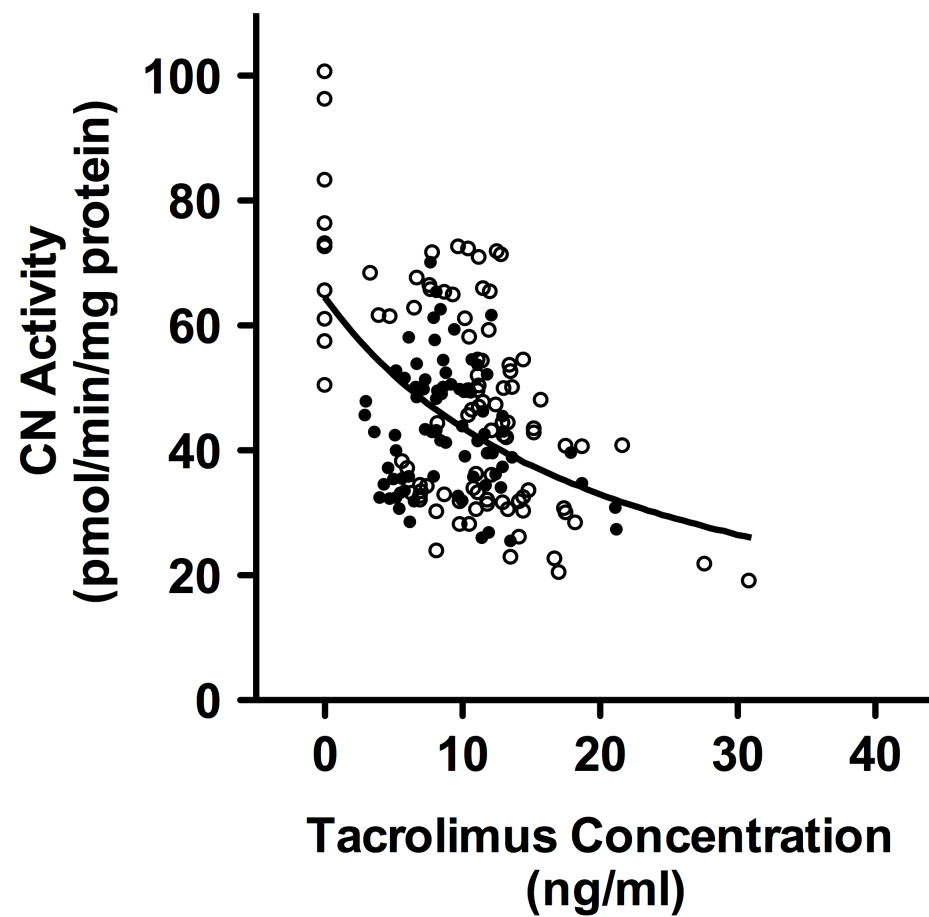


Fig. 5

